European Institute of Knowledge & Innovation

Annals of Innovation in Medicine (AIM) ISSN: 2977-0335

Research Article

Effect of Exposure to Air Freshener on Hepatic Enzymes and Haematological Parameters of Albino Rats

Onengiyeofori Ibama[1], Onengiye Davies-Nwalele[2], Arit Okechukwu Nwogu[3], Abiye Chiladi Isomah[3]

1. Faculty of Medical Laboratory Science, Rivers State University, Nkpolu, Port-Harcourt Nigeria
2. Faculty of Medical Laboratory Science, Federal University Otuoke, Nigeria.
3. College of Medical Sciences, River State University, Nigeria

*Correspondence: Onengiyeofori Ibama (onengs4u@yahoo.com)

https://doi.org/eiki/10.59652/aim.v2i3.232

Abstract:

Air fresheners, which are available as incense sticks, scented candles, aerosols, liquids, gels, and electric diffusers, are used to mask odors with refreshing scents. They are widely used in homes, cars, and offices, marketed to create a clean and pleasant indoor atmosphere. However, many air fresheners contain volatile organic compounds (VOCs), which might have harmful effects on human health. This study evaluated the effects of exposure to a gel air freshener on hepatic enzymes and hematological parameters in albino rats. Thirty (30) albino rats, weighing 200-260g, were divided into three groups of ten rats each, and acclimatized for two weeks under 12-hour light/dark cycles with free access to food and water. Group I (Control) was not exposed to the air freshener, Group II was exposed to the air freshener for 4 hours daily for 28 days, and Group III for 8 hours daily for 28 days. After the treatment, the rats fasted overnight, were anesthetized with chloroform, and blood samples were collected via cardiac puncture. AST and ALT were assayed using enzymatic methods, and hematological parameters were measured with the Sysmex XP-300 Automated Haematology Analyzer (5-part). Results showed a significant increase ($p<0.05$) in AST, ALT, hematocrit, hemoglobin, red blood cells, and platelet levels in Group III compared to Groups I and II. No significant difference was found between Groups I and II. This suggests that 8-hour daily exposure to air freshener for 28 days induces hepatocellular damage and alters hematological parameters in albino rats, with the extent of damage increasing with longer exposure. However, further studies on humans are recommended.

Keywords: Air fresheners, hepatic enzymes, haematological parameters, aspartate aminotransferase, alanine aminotransferase, volatile organic compounds, inhalation exposure, liver damage

Received: 24 June 2024
Accepted: 5 July 2024
Published: 7 July 2024

1. Introduction

The adage "cleanliness is next to godliness" highlights the importance of a clean and pleasantly scented home, which exudes warmth and welcome. However, daily activities like cooking and pet care can result in lingering unpleasant odors. Air fresheners are commonly used to mask these odors, promoting a pleasant environment (1); (2). These products are prevalent in various indoor spaces, including offices, schools, hospitals, homes, and transportation modes, enhancing hygiene and sensory appeal. Despite their popularity, research indicates that air fresheners contribute significantly to indoor air pollution, posing health risks (3).

Air fresheners, which come in forms like incense sticks, scented candles, aerosol sprays, and electric diffusers, eliminate odors through chemical reactions or masking scents (3). However, they increase the number of odorous compounds rather than reducing air pollutants, often releasing over a hundred different chemicals, including volatile organic compounds

(VOCs) such as terpenes, benzene (4), formaldehyde, and phthalates (5), toluene (6), linalool (7). Despite their claims of creating a clean and sweet-smelling environment, many air fresheners contain harmful ingredients (2). These fumes when inhaled may trigger an immune reaction that involves the activities of blood cells and the detoxification function of the liver (1); (8).

These products release compounds linked to cancer, neurotoxicity, and endocrine disruption, thus affecting indoor air quality and potentially leading to health issues like eye, nose, and throat irritation, headaches, and damage to the liver, kidneys, and central nervous system (9); (2). Even at levels below accepted safety standards, chemical exposures from air fresheners can increase the risk of respiratory problems, such as asthma in children, and cause symptoms like dizziness and irritated eyes (2).

Figure 1: Some Brands of Air Refreshers

The liver, which is essential for nutrient metabolism and waste excretion, detoxifies foreign molecules and substances to reduce their toxicity (10), promoting their excretion through the intestines or kidneys. The critical role of the liver is evident in its ability to cause death if its functions are lost (11).

There is a scarcity of data on the evaluation of hematological parameters and hepatic enzymes following air freshener exposure. The only comparable study, conducted by (12), reported significantly elevated hepatic indices in rats exposed to air fresheners compared to the control group. Thus, conducting this study is essential to gain a deeper understanding of the changes in these parameters resulting from air freshener exposure. This study was aimed at evaluating the effect of exposure to air freshener on some liver enzymes and haematological parameters in albino rats.

2. Materials and Method

2.1 Purchase of Air fresheners and Test Kits

A brand of gel air freshener, named Sunshine air freshener, was randomly selected and purchased from Mile 3 Market in Port Harcourt, Rivers State, Nigeria. The air freshener was coded and stored at room temperature. The name, active ingredients, and expiry dates were recorded from the packages. Rat-specific test kits for AST and ALT, produced by Bioassay Technology Laboratory in Shanghai, China, were obtained from a local sales representative in Nigeria.

2.2 Ethical Considerations

The internationally accepted National Institutes of Health (NIH) Guide for Care and Use of Laboratory Animals were observed.

2.3 Experimental Animals

A total of 30 albino rats (both male and female), weighing between 200-260 grams, were sourced from the animal house at the Faculty of Pharmacy, University of Port Harcourt, Rivers State, for this study. The rats were kept in well-ventilated cages, maintained at a temperature of 27-30°C, with a 12-hour light/dark cycle. They had free access to tap water and dry rat pellets (bought at Mile 3 Market, Port Harcourt). The rats were randomly assigned to three groups of 10 rats each, and were allowed to acclimate for 28 days before the experiment began. After the animals adjusted to their surroundings, the experiment began. The first group (the control group) was not exposed to the air freshener, while the second and third groups (the test groups) were. The second group was exposed to the air freshener for 4 hours a day, every day for 28 days, while the third group was exposed for 8 hours a day, also for 28 days

2.4 Blood Sample Collection

After the 28 days of exposure to the air fresheners, the albino rats were allowed to fast overnight, after which they were anaesthetized in a jar containing cotton wool soaked in chloroform to render them unconscious. In this unconscious state, they were quickly removed from the jar, and 7ml of whole blood specimen was collected (using a sterile syringe and needle) through cardiac puncture into sterile sample containers; 4 ml was dispensed into plain bottles, which was spun at 3500rpm for 5 minutes to obtain the plasma which was in turn, used to analyze for AST and ALT using the rat-specific test kits, while the remaining 3ml of the blood specimen was dispensed into EDTA-anticoagulated bottles and was used to analyze for haematological parameters.

2.5 Sample Analysis

Enzymatic methods were used to measure the serum levels of AST and ALT (13). Hematological parameters were analyzed using the Sysmex XP-300 Automated Hematology Analyzer (5-part).

2.6 Statistical Analysis

The data generated from the analysis were expressed as Mean ± Standard Deviation and analyzed using the Statistical Package for Social Sciences (SPSS) version 26. Comparisons of mean and standard deviation values for various parameters between the test and control groups were made using one-way ANOVA and Tukey tests. Results were considered statistically significant at a 95% confidence interval ($p<0.05$).

3. Results

3.1 Comparison of the Levels of AST and ALT of Group I (Control), Group II, and Group III

Details of the comparison of the mean AST and ALT levels of the control (group I) and test (group II and group III) are shown in Table 3.1. The mean AST levels of group I, group II, and group III are 72.22±2.89 U/L, 71.07±7.21 U/L, and 89.20±10.85 U/L respectively, with the mean AST level of group III being significantly higher ($p=0.00001$) when compared with the mean levels of group I and group II. Similarly, the mean ALT levels of group I,

group II, and group III are 79.56±2.22 U/L, 89.90±12.52 U/L, and 108.65±11.06 U/L respectively, with the mean ALT level of group III being significantly higher (p=0.00001) when compared with the mean levels of group I and group II.

Table 3.1: Mean Levels of AST and ALT for Group I, Group II, and Group III Compared

GROUPS	AST (U/L)	ALT (U/L)
Group I (Control)	72.22±2.89	79.56±2.22
Group II	71.07±7.21	89.90±12.52
Group III	89.20±10.85[ab]	108.65±11.06[ab]
F-value	17.361	22.968
P-value	0.00001	0.00001
Remark	SS	SS

Key: SS – statistically significant, AST -Aspartate transaminase, ALT - Alanine transaminase, [a] – significantly different from Group I, [b] – significantly different from Group II

3.2 Comparison of the Levels of Haematological Parameters of Group I (Control), Group II, and Group III

Table 3.2 compares the mean levels of hematological parameters among the control group (group I) and the test groups (group II and group III). The mean WBC levels were similar across all groups, with no significant difference (p=0.400). However, group III showed significantly higher mean levels for several parameters compared to groups I and II. HCT: Group III (51.60±3.50%) was significantly higher (p=0.00001) than group I (40±4.22%) and group II (40.20±3.23%). HB: Group III (16.99±1.17 g/L) was significantly higher (p=0.00001) than group I (13.05±1.49 g/L) and group II (13.19±1.19 g/L). RBC: Group III (6.19±0.36 x10^{12}/L) was significantly higher (p=0.00001) than group I (4.31±0.53 x10^{12}/L) and group II (4.61±0.77 x10^{12}/L). Platelets: Group III (194±10.34 x10^{3}/µl) was significantly higher (p=0.030) than group I (182.90±7.40 x10^{3}/µl) and group II (182.70±13.14 x10^{3}/µl).

Table 3.2: Mean Levels of Some Haematological Parameters for Group I, Group II, and Group III Compared

GROUPS	WBC (X 10^{3}/µl)	HCT (%)	HB (g/l)	RBC X 10^{12}/L	PLATELET (X 10^{3}/µl)
Group I (Control)	5.66±0.41	40±4.22	13.05±1.49	4.31±0.53	182.90±7.40
Group II	5.65±0.28	40.20±3.23	13.19±1.19	4.61±0.77	182.70±13.14
Group III	5.86±0.44	51.60±3.50[ab]	16.99±1.17[ab]	6.19±0.36[ab]	194±10.34[ab]
F-value	0.949	32.707	30.008	30.512	4.025
P-value	0.400	0.00001	0.00001	0.00001	0.030
Remark	NS	SS	SS	SS	SS

Key: SS – statistically significant, NS – Not significant, WBC -White blood cell, HCT - Haematocrit, HB – Haemoglobin, RBC – Red blood cell, a – significantly different from Group I, b – significantly different from Group II

3.3 Correlation between Duration of Exposure and Liver Enzymes

Table 3.3 shows the correlation between the duration of exposure and liver enzyme activity: AST: A significant positive correlation (correlation coefficient = 0.627, p = 0.0002). ALT: A significant positive correlation (correlation coefficient = 0.783, p = 0.0002).

Table 3.3: Correlation between Duration of Exposure and Liver Enzymes

	Duration of Exposure (Years) vs. AST	**Duration of Exposure (Years) vs. ALT**
Pearson r	0.627	0.783
P value	0.0002	0.0002
Remark	SS	SS

Key: SS – statistically significant, NS – Not significant, AST -Aspartate transaminase, ALT - Alanine transaminase.

3.4 Correlation between Duration of Exposure and Haematological Parameters

Table 3.4 presents the correlation between the duration of exposure and hematological parameters. The findings are as follows: WBC: A nonsignificant positive correlation (correlation coefficient = 0.216, p = 0.251). HCT: A significant positive correlation (correlation coefficient = 0.735, p = 0.000004). HB: A significant positive correlation (correlation coefficient = 0.732, p = 0.000004). RBC: A significant positive correlation (correlation coefficient = 0.775, p < 0.001). Platelets: A significant positive correlation (correlation coefficient = 0.411, p = 0.024).

Table 3.4: Correlation between Duration of Exposure and Haematological Parameters

	Duration (Years) vs. WBC	**Duration (Years) vs. HCT**	**Duration (Years) vs. HB**	**Duration (Years) vs. RBC**	**Duration (Years) vs. PLATELET**
Pearson r	0.216	0.735	0.732	0.775	0.411
P value	0.251	0.000004	0.000004	<0.001	0.024
Remark	NS	SS	SS	SS	SS

Key: SS – statistically significant, NS – Not significant, WBC -White blood cell, HCT - Haematocrit, HB – Haemoglobin, RBC – Red blood cell

4. Discussion

This study aimed to assess the effects of exposure to a gel air freshener on liver enzymes (AST and ALT activities) and haematological parameters (white blood cells, haematocrit, haemoglobin, red blood cells, platelets) in albino rats. A significant increase in AST and ALT levels was observed in the group exposed to air fresheners for 8 hours daily (group III) for 14 days compared to the group exposed for 4 hours daily (group II) and the control group (group I) not exposed to air fresheners. Since AST and ALT are markers of hepatocellular injury, the results suggest that prolonged exposure to this air freshener may induce liver damage in rats, which may be attributed to the volatile organic compounds (VOCs) they contain

(14). These findings align with those of (15) and (1), who also reported elevated AST and ALT levels in albino rats exposed to air fresheners.

There was no significant difference in total white blood cell (WBC) count between the groups exposed to air fresheners (groups II and III) and the control group (group I). Air fresheners are known to contain VOCs (16), which are inhaled by the rats. This result suggests that WBC levels were not affected by these VOCs, contradicting previous studies that reported elevated WBC levels due to VOC exposure (17). However, the findings of this study contrast with those of (18), who reported a significant decrease in WBC levels in rats exposed to air fresheners. This discrepancy could be due to the use of liquid air freshener in their study, whereas our study employed a gel air freshener.

The rats exposed to air fresheners for 8 hours daily (group III) also showed elevated levels of haematocrit, haemoglobin, and red blood cells compared to the other groups. This increase might be due to higher erythropoietin production in response to hypoxia (19), indicating that air fresheners can reduce oxygen delivery to tissues, triggering a response to increase red blood cell production to compensate for the oxygen deficit. Furthermore, the platelet count was higher in the group exposed to air fresheners for 8 hours daily (group III), suggesting that VOCs might induce inflammation or other conditions leading to increased thrombopoietin production (16). The findings of this study also contrast with those of (18), who reported a significant decrease in the levels of red blood cells, haematocrit, and haemoglobin in rats exposed to air fresheners. Again, this discrepancy could be due to the use of liquid air freshener and rabbits in their study, whereas our study employed a gel air freshener.

The study also found a significant positive correlation between the duration of exposure to air fresheners and hepatic enzyme levels (AST and ALT), indicating that longer exposure leads to greater liver damage. Conversely, there was a nonsignificant positive correlation between exposure duration and WBC count, implying no effect on WBC levels. However, there was a significant positive correlation between exposure duration and other haematological parameters (haemoglobin, haematocrit, red blood cells, and platelets), suggesting that longer exposure increases these parameters.

5. Conclusion

This study demonstrates that exposing albino rats to a gel air freshener for 8 hours daily over 28 days resulted in elevated levels of hepatic enzymes AST and ALT, suggesting hepatocellular necrosis. In contrast, a 4-hour daily exposure for the same period did not significantly affect these enzyme levels. Regarding haematological parameters, the exposure did not alter total white blood cell levels. However, hemoglobin, hematocrit, red blood cells, and platelets levels were elevated after 8 hours of daily exposure for 14 days. Conversely, a 4-hour daily exposure for 28 days did not induce changes in these parameters. The study also reveals that increased duration of exposure to the air freshener correlates with higher AST and ALT levels, indicating greater hepatocellular damage. Additionally, longer exposure durations led to increased levels of hemoglobin, hematocrit, red blood cells, and platelets, indicating alterations in these hematological parameters.

References

1. Airaodion AI, Ogbuagu EO, Atiba FA, Olawoyin DS, Alabi OJ. Frequent exposure to air-freshener reduces male fertility. *Asian Research Journal of Gynaecology and Obstetrics*. 2020a;3(3):19-31.
2. Wickramasinghe AP, Karunaratne DGGP, Sivakanesan R. PM10-bound polycyclic aromatic hydrocarbons: biological indicators, lung cancer risk of realistic receptors and 'source-exposure-effect relationship' under different source scenarios. *Chemosphere*. 2012;87(11):1381-1387.
3. Kim S, Hong SH, Bong CK, Cho MH. Characterization of air freshener emission: the potential health effects. *The Journal of Toxicological Sciences*. 2015;40(5):535-550.
4. Smith MT. Advances in understanding benzene health effects and susceptibility. *Annual Review of Public Health*. 2010;31:133-148.
5. Steinemann AC. Fragranced consumer products and undisclosed ingredients. *Environmental Impact Assessment Review*. 2009;29(1):32-38.
6. Ono A, Kawashima K, Sekita K, Hirose A, Ogawa Y, Saito M, et al. Toluene inhalation induced epididymal sperm dysfunction in rats. *Toxicology*. 1999;139(3):193-205.

7. Aprotosoaie AC, Hăncianu M, Costache II, Miron A. Linalool: a review on a key odorant molecule with valuable biological properties. *Flavour and Fragrance Journal*. 2014;29(4):193-219.
8. Nørgaard AW, Kudal JD, Kofoed-Sørensen V, Koponen IK, Wolkoff P. Ozone-initiated VOC and particle emissions from a cleaning agent and an air freshener: risk assessment of acute airway effects. *Environment International*. 2014;68:209-218.
9. Mohammed B, Yakasai UA. Subacute toxicity study of some brands of air fresheners sold in Kano on Swiss albino rats (Rattus norvegicus). *Intl J Adv Acad Res| Sci, Technol & Engineer*. 2017;3:19-32.
10. Ibama O, C. Amadi F. Assessment of Serum Levels of Some Heavy Metals in Carpenters Residing in Port-Harcourt in Relation to Their Lifestyle. *Asian Journal of Research in Medical and Pharmaceutical Sciences*. 2018;4(4):1-7.
11. Ozougwa J, Eyo JE. Hepatoprotective effects of Allium cepa (onion) extracts against paracetamol-induced liver damage in rats. *African Journal of Biotechnology*. 2014;13:2679-2688.
12. Airaodion AI, Olawoyin DS, Alabi OJ, Atiba FA, Ogbuagu EO. Air freshener induced oxidative stress and its adverse effects on immunity. *International Journal of Health, Safety and Environment*. 2020b;6(5):579-587.
13. Reitman S, Frankel S. A colorimetric method of determination of serum oxaloacetate and glutamate transaminase. *Journal of Clinical Pathology*. 1957;28:56-58.
14. Cheville NF. Introduction to Veterinary Pathology. 3rd ed. Iowa State University Press; 2006. pp. 6-42.
15. Yakasai AU, Mohammed B. Biochemical effects of gel and liquid air fresheners sold in Kano on Swiss albino rats (Rattus Norvegicus). *Bayero Journal of Pure and Applied Sciences*. 2022;13(1):554-559.
16. Kim S, Lee AY, Cho MH. Inhaled exposure to air fresheners aggravated liver injury in a murine model of nonalcoholic fatty acid liver disease. *Heliyon*. 2021;7(3):1-7.
17. Akpan KV, Sogbanmu TO, Otitoloju AA. Effects of volatile organic solvents' inhalation on haematological and histological indices of Mus Musculus. *Current Advances in Environmental Science*. 2014;2(2):46-51.
18. Zuhair YA1-S. Hematological and biochemical effects of an air freshener in rabbits. *Journal of American Science*. 2012;8(8):70-73.
19. Ashade OO, Igbokwe L. Haematological and biochemical assessment of composite wood extracts in albino rat (Male Wister Strain). *Journal of Natural Sciences Research*. 2014;4(18):2224-3186.